THE SUSCEPTIBILITY OF CELLS TO RADIUM RADIATIONS.

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It has frequently been observed that cells may show at different periods of their existence marked variations in susceptibility to radium radiations. An embryonic cell is more quickly injured than the same cell in the adult condition: at the metaphase, cells are more sensitive than they are immediately before or after that brief period (1). Bohn (2) first suggested that the underlying cause of such differences in response must be sought in the physiological condition of the cells at the time of radiation. That they cannot be due to changes in the absorptive power of protoplasm is obvious: whether a cell is sensitive or not, the rays are absorbed to the same extent. The actual changes produced by them in protoplasm must therefore be the same. But the reaction of the cell to such changes differs immensely. We may therefore say that a cell is susceptible when it is in such a physiological condition that a modification produced by the rays results in a greater or less injury, and that it is resistant when the same modification is not followed by injurious effects.

The object of the present paper is to show that among the conditions which affect the susceptibility of cells to radium radiations are (1) the temperature of the cells at the time of exposure, and (2) the relative permeability of the surface layer of the cell.

The experiments to be described were carried out on certain Protozoa, for these cells are better adapted to this kind of experimentation than any others. They can live in both high and low temperatures without injury: and different genera show marked differences in permeability. Cells of the same species, even descendants of the same individual, vary widely in their reaction to radium radiations at different periods of their life cycle. *Paramœcium* is perhaps the best cell for experimental purposes since it is more susceptible than any other common type.

I will not here review the results obtained by other investigators who have tested the effects of radium radiations and X-rays on Protozoa. Different methods of exposing the cells lead to such wide variations in results that comparisons cannot safely be made. In general, however, cells which are sensitive to the one are also sensitive to the other type of radiation. Great differences in the susceptibility of cells of the same species have been reported. Thus Zuelzer (3) states that *Pelomyxa palustris* when exposed to 6 mg. of radium element dies sometimes within one hour and sometimes only after four hours of continuous exposure. Such differences make any conclusion as to the length of the lethal dose out of the question. But with appropriate methods the lethal dose is found to be constant.

Methods.

The method used in the following experiments is this. The radium was enclosed in a glass capsule which prevented the alpha rays from escaping. In the experiments on the relation of temperature and permeability to susceptibility, the strength equalled 13.4 mg. of element. In the third series, on the change in permeability induced by the radiations, the strength was 25 mg. of element. The radium tube was used unscreened and was supported above the drop of culture medium at a distance of 2 mm. Thus all of the rays which emerged from the lower side of the tube could reach the cells. The whole preparation was kept in a moist chamber at the desired temperature.

In order to determine what type of rays produced the effects which are to be described, I interposed between the radium tube and the *Paramœcia* lead sheets of various thicknesses, thus filtering out the more penetrating rays. All of the beta rays are stopped by 2 mm. of lead: the gamma rays are not affected. It became apparent at once that the changes produced in the *Paramœcia* were due to the action of the slowest beta rays, for when a lead screen of 0.12 mm. was interposed the *Paramœcia* were affected hardly at all. This is to be expected, for the surface layer of the cells is very thin and can absorb only those rays which have a low velocity: it offers almost no resistance to rays having considerable powers of penetration.

In conducting these experiments it was found necessary to use

only *Paramœcia* from a pure culture, for unrelated wild cells show great variations in their susceptibility to the rays. Another necessary condition has been mentioned by Jacobs (4), namely, that in each test the same amount of liquid must be used.

The Reaction of Paramœcium to Radium Radiations.

When a *Paramœcium* is exposed to radium radiations under the conditions described, it quickens its movements at first and then gradually slows down and ceases to swim unless the dish is shaken. Later the contractile vacuoles pulsate more and more slowly and finally stop, usually in the expanded condition. If radiation is longer continued, a typical cytolysis ensues. The cells imbibe water, swelling considerably in consequence, and the ectoplasm bulges out in the form of clear vesicles which later run together. Then the pellicle separates from the rest of the cell carrying with it the cilia. The protoplasm is now highly fluid. At this time the macronucleus, in stained preparations, is seen to be divided into several parts. Not infrequently the cells burst violently. These phenomena are in every point similar to those which are observed when *Paramœcium* is treated with a variety of cytolytic agents, as described by Budgett (5), Harvey (6), and Jacobs (4).

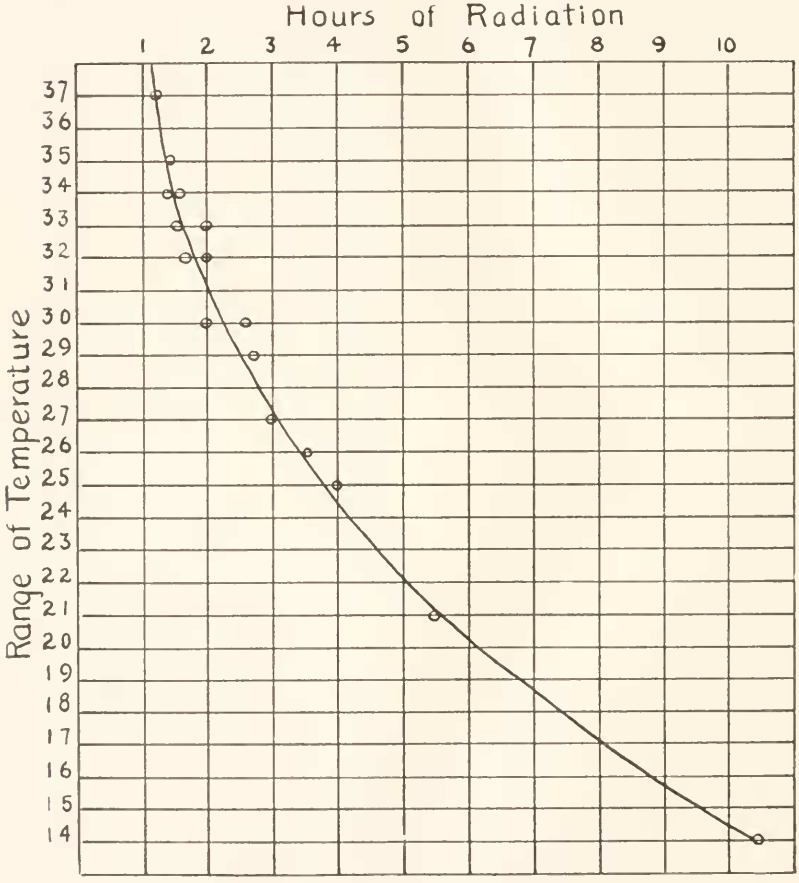
The Relation of Temperature to Susceptibility.

Rhodenburg and Prime (7) first pointed out that there is a definite correlation between temperature and the susceptibility of cells when treated with X-rays. In their experiments they exposed mouse sarcoma in vitro at a temperature of 42° C. to a definite dose of X-rays, and then inoculated healthy mice with the radiated cells. At this temperature 10 per cent. of the inoculations failed to take. When the cells were radiated at 43° C., 76 per cent. of the inoculations failed. Control experiments proved that these temperatures alone are not sufficient to produce this effect. The combination of high but sublethal temperatures with radiation was five times as effective as radiation alone.

Mammalian tissue cannot be subjected to wide variations in temperature, but the Protozoa can live normally at temperatures as low as 15° C. and as high as 37° C. In the following experiments these were the limits employed.

When *Paramœcia* are radiated at high temperatures, they suc-

cumb much more quickly than at low temperatures. The results are shown in the accompanying figure. An analysis of the curve shows that for each increase of about 8° C. the length of the lethal dose is halved. The curve is thus similar to that which expresses the relation between temperature and the velocity of a great number of reactions both inorganic and physiological.



TEXT-FIGURE 1. The effect of temperature on susceptibility.

Snyder (8) cites more than fifty physiological reactions which conform to this type of curve: Woodruff and Baitsell (9) show also that the division rate of *Paramœcium aurelia* varies similarly with changing temperature.

The cells were not injured by these temperatures alone. While

the experiments were in progress the room temperature varied from 30° to 36° C. and the cultures flourished. Single lines of cells showed a steady division rate of about 2½ divisions per day. In the coolest temperatures the cells remained normal, and regained the usual division rate on being brought back into a warmer place.

This increase in susceptibility at high temperatures is not due to any increased activity of the radiations, nor to any change in the power of protoplasm to absorb the rays. The amount of radiation absorbed is determined by the atomic constitution of protoplasm and this does not vary materially during changes in temperature. One of the conditions which varies with the temperature is the permeability of the cell membrane. Hober (10) states that the permeability of plant cells is doubled with each increase of 10° C., the cells being eight times as permeable at 30° as they are at 0° C. That *Paramœcia* are more permeable in warm than in cold solutions can be demonstrated by staining them at 30° and 14° C. in neutral red. In the higher temperature they stain deeply: at the lower, they do not stain at all. Whether a change in permeability is the only cause of increased sensitiveness to radiations remains to be demonstrated. That it is an important factor is shown in the next section.

The Relation of Permeability to Susceptibility.

The fact that cells are most sensitive to radiations when their permeability is increased by heat suggests that the two phenomena are related. Here again the Protozoa are admirably adapted for testing this point, for the permeability of the cell membrane can be measured in the living condition. The method employed in these experiments is that followed by Harvey (6). A number of *Paramœcia* from a pure culture are drawn up into a capillary pipette which is calibrated so that exactly the same amount of liquid is taken in each experiment. The cells are stained for ten minutes in a solution of 0.02 per cent. neutral red mixed with 10 cc. of tap water. At the end of this time the vacuoles at the posterior end of the cells are a bright pink. The surrounding protoplasm is also colored. The neutral red in this dilution is not toxic, although in more concentrated solutions it produces cytolysis.

The cells are now drawn up in a calibrated pipette and added

to exactly 2 cc. of $n/1280$ NH_4OH solution. The amount of liquid thus added reduces the strength of the ammonia to $n/1300$. The stained *Paramœcia* when put into this solution give first the avoiding reaction and quickly begin to lose color. The protoplasm, and later the gastric vacuoles, turn yellow, and finally become colorless. There is a wide variation in the rate at which the color fades in individual cells, some destaining in three or four minutes while others retain some pink color as long as ten minutes. In order to determine the average time for destaining the usual number of cells (about 40), I used the following method. The cells were observed, during their destaining, under a binocular microscope and each one, as soon as it lost color, was removed and the time which had elapsed since its first entrance into the ammonia solution recorded. The following measurements, typical of many, indicate the rate of destaining.

PARAMŒCIUM DESTAINED IN $n/1280$ NH_4OH.

Minutes Elapsed.	No. of Cells Destained.
1	0
2	0
3	0
4	3
5	2
6	4
7	5
8	8
9	6
10	6
11	6
12	5
Total	45 Ave. 8.5 min.

By this method the personal equation is greatly reduced since the observer cannot form any idea of what the average will be until the entire number of cells has been destained. Many tests on the same pure culture of *Paramœcium* gave very constant results, the average time of destaining in tests carried out on the same day varying less than one half minute. Other lines of *Paramœcia* showed somewhat different averages but even here they differed from each other by not more than one and one half minutes.

A study of *Paramœcium* cells at different phases of their life cycle shows that the permeability varies, being much greater at the time of conjugation than at any other period. Thus among four lines of cells in which conjugation did not occur, the de-

staining time varied from 7.8 minutes to 9 minutes. In a culture which was undergoing an epidemic of conjugation the pairs showed an average destaining time of 4.5 minutes. This culture was presumably not pure, and the pairs in their reaction to the ammonia showed a wider variation than was found in any homogeneous group. But the difference in permeability between conjugants and non-conjugants was in every case large enough to be significant.

Other Protozoa differ from *Paramœcium* in permeability. As Harvey has pointed out, *Stylonichia* and *Oxytricha* are comparatively impermeable. Indeed, after ten minutes in neutral red of the usual concentration they have taken up almost no color at all. The stain must act for twenty-five to thirty-five minutes before these cells are stained sufficiently for experimental purposes, and even after this time they are not as highly colored as *Paramœcium* is after ten minutes. The time required for destaining *Stylonichia* varies somewhat in different cultures, but the average is approximately forty minutes.

When the relative permeability of these cells is compared with the length of their lethal dose of radium radiations, we find a close correlation between the two measurements: that is, cells which are relatively permeable are quickly killed by the rays, while those which are less so are more resistant. The following table indicates these relations.

COMPARISON OF THE DESTAINING TIME AND LETHAL DOSE OF RADIUM RADIATIONS AT 27° C.

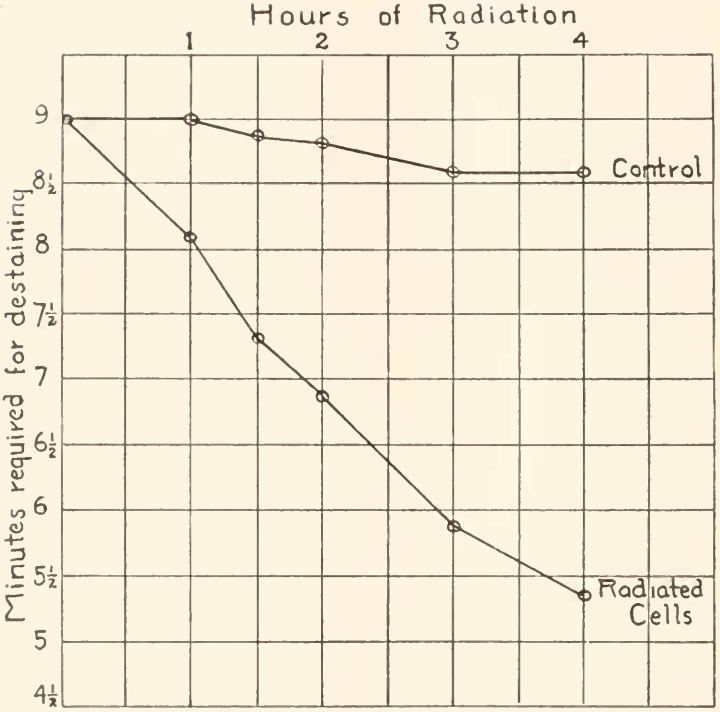
	Destaining Time.	Lethal Dose.
Paramœcium single cells	8.6 min.	3 hours
Paramœcium conjugating	4.5 min.	1½ hours
Stylonichia	40. min.	15 hours

It is evident therefore that the susceptibility of these Protozoa to radium radiations varies directly with the permeability of the surface layer of the cell.

THE EFFECT OF RADIUM RADIATIONS ON THE CELL MEMBRANE.

The question naturally arises, what is the reason for this correlation? The answer is to be found in the fact that the rays which are absorbed produce in the cell membrane changes which lead to increased permeability. The experiments described below indicate the rate at which these changes take place.

In the following experiments the radium amounted to 25 mg. of element. The cells were exposed in the same manner as before, then stained in neutral red and destained in $n/1280$ NH_4OH. The accompanying figure shows the results of experiments per-



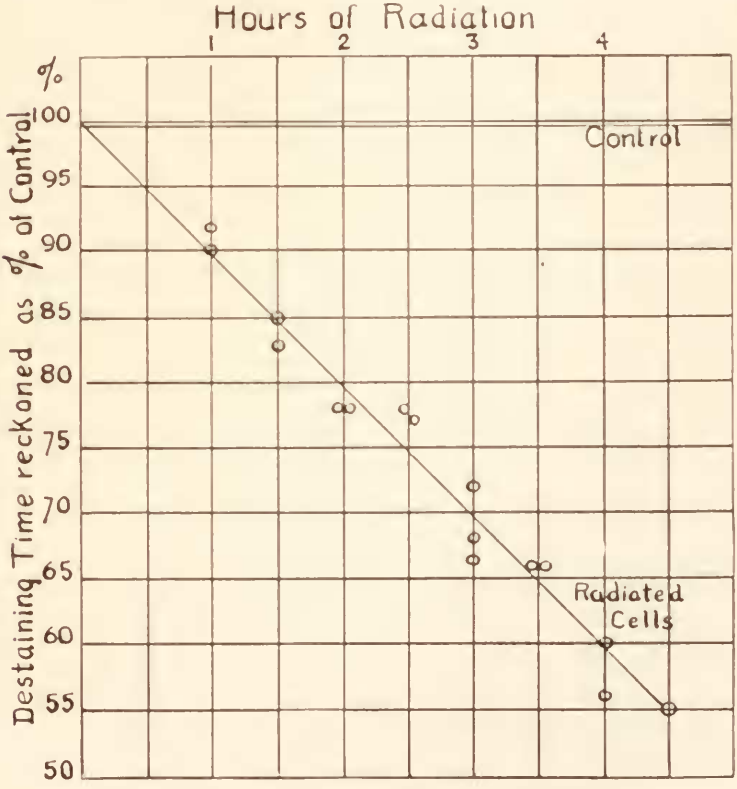
TEXT-FIGURE 2. The effect of radiations on permeability.

formed in one day. It is necessary to make many tests within the limits of a few hours for the cells vary somewhat in their reactions to these manipulations with changing conditions of food, etc. By using two tubes, each amounting to 25 mg. of element it was possible to perform two experiments simultaneously and thus make seven or eight determinations in a single day. The temperature in these tests remained uniformly at 22° C.

The control cells showed a slight increase in permeability after remaining in a small drop of culture medium for some hours. This however is not significant, for cells left as long as eight hours show practically the same reaction to ammonia as those

left for only two hours in a small drop. But the radiated cells display a marked shortening in the time required for destaining, or, in other words, they show a considerable increase in permeability, the change beginning very soon after the exposure to radium commences. At the end of four hours the cells are still alive but are for the most part motionless. If the exposure is longer continued the cells die, showing the characteristic signs of cytolysis.

The second figure shows the result of a larger number of tests.



TEXT-FIGURE 3. The effect of radiation on permeability.

Since they were made during the course of many weeks the controls varied somewhat, although on any one day they were very uniform. The time required for destaining is here given in percentages of the control time which is reckoned as 100 per cent. The curve clearly indicates that the normal semi-permeability of

Paramœcium increases progressively as the exposure is more and more prolonged.

Some of the radiated cells were removed to separate drops of culture medium before treatment with the stain, and their division rate observed. Those which were exposed for one hour or a little less frequently showed a higher rate than the controls. This result has been described by Markowits (11) who studied the effect of mesothorium rays on *Paramœcium*. The acceleration can be observed for five or six generations, after which the cells return to normal and show no evidences of injury. The same phenomenon I have observed in sea urchin eggs when lightly radiated (12). Indeed, a stimulation in the rate of growth is of general occurrence, having been noticed in the case of growing plants, embryos, tissue cultures, and abnormal tissue growths.

From these facts we may conclude that the slowest beta rays increase the permeability of the surface layer of *Paramœcium* cells. If the exposure is brief, this change in permeability is accompanied by an acceleration in the rate of cell division. If it is more prolonged, a destructive cytolysis ensues, and the cells die.

Discussion.

Radiations which are absorbed at the surface of the cell produce definite changes which lead to an increase in permeability, and, if the exposure is sufficiently prolonged, to complete cytolysis. For a definite dose, such changes must be the same in extent, regardless of the physiological condition of the cell, for the absorptive power of protoplasm remains constant. Yet it is quite apparent that a given dose of radiation may result in no appreciable injury in certain instances, while in others it is followed by the death of the cells. This is clearly shown in the text-figures. For example, at 22° C. radiated *Paramœcia* undergo cytolysis, under the conditions described, in five hours. From the beginning of the exposure to the death of the cell there is a steady increase in permeability. An exposure of half this duration is followed by no permanent injury. But when cells are radiated at 30° C. they are cytolyzed after only two and one half hours. Cytolysis occurs when the permeability of the cell has been raised above a definite limit. If it is already high, due to high temperature or to other conditions, the cytolytic action of

the rays quickly raises the permeability above the limit and the cell dies. But if it is low, the lethal point is reached only after a prolonged exposure.

A similar phenomenon is observed in eggs exposed during different phases of mitosis. At the metaphase their permeability, as shown by Lyon (13) and others, is notably greater than at any other period: so also is their susceptibility. I have shown (12) that an amount of radiation which will induce a quickened cell division in sea urchin eggs, when applied just before or after the metaphase, has a retarding effect when applied at that period. That is, the cells are more sensitive then than they are during the prophase or telophase of mitosis.

The same results are obtained when other cytolytic agents are used in place of radium radiations. Lillie (14) finds that if freshly fertilized sea urchin eggs, which are highly impermeable, are treated with hypotonic sea water they resist its cytolytic action for thirty minutes. But if they are placed in this solution when the cleavage furrow appears, they rapidly undergo cytolysis.

It appears possible therefore that the susceptibility of cells may be raised by the simple expedient of increasing their permeability by heat or by some other means. This has been done by Rhodenburg and Prime (7) in the experiments already cited. How far this method can be used in the treatment of abnormal tissue growths remains to be demonstrated.

The fact that agents which differ so widely as do radium radiations and hypotonic sea water produce the same effects under similar conditions suggests that the action of these rays, and also other types of radiant energy, such as ultra-violet light and alpha rays, is not peculiar to themselves. Indeed it may be said that any of the rays which are absorbed at the surface of the cell will cause changes differing in no way from those produced by a great variety of chemical cytolytic agents.

The more penetrating beta rays, the gamma, and X-rays appear to be so slightly absorbed at the surface of cells which are freely exposed to them that they can produce little or no effect. Richards (15) tested the permeability of various eggs and of *Arenicola* larvæ after an exposure to X-rays and found no evidence of any increase. I have shown that the rapid beta and the gamma rays of radium do not act on the surface layer of *Paramœcium*.

But it has been demonstrated that very definite proportions of these rays are actually absorbed by cells of deep-lying tissues. And histological study shows that such cells after radiation imbibe water, swell considerably, and undergo degenerative changes which have all the appearance of a typical cytolysis. It is apparent, then, that any kind of rays which are absorbed produce the same effects. But these more penetrating rays differ from the less penetrating types in this respect, that they are also able to bring about degenerative changes in the interior of the cell, particularly in the nucleus, which the latter, because of their slight penetrating power, are unable to produce.

I have mentioned the fact that a brief radiation with the slow beta rays produces an acceleration in the division rate of *Paramœcium*. This effect is not restricted to the action of these particular rays, for all the radiations of radium, as well as X-rays, can produce this result if the proper exposure is made. This has been demonstrated in a great variety of cases, some of which have already been cited. According to Lillie (16) such acceleration is the direct outcome of increased permeability, for this condition allows a freer interchange of CO_2 and Ó through the surface layer and a consequent hastening of all metabolic activities. It may be possible, therefore, that all cases of stimulation following exposure to radium radiations and to X-rays can be explained on this basis.

Summary.

1. The susceptibility of *Paramœcium* to radium radiations (chiefly the slowest beta rays) varies with the temperature at the same rate as do physiological reactions of various kinds.

2. The susceptibility also varies directly with the degree of permeability of the surface layer of the cell.

3. The slow beta rays act on the surface layer of the cell, increasing its permeability, and if allowed to act long enough, causing a typical cytolysis. In this respect they resemble other types of radiant energy, and diverse chemical cytolytic agents.

4. Cells which have a relatively high permeability are more susceptible than those having low permeability, for the cytolytic action of the rays is quickly followed in the former by a cytolysis which is irreversible, while in the latter it is reversible.

5. It is suggested that this increase in permeability, following brief exposures, is the cause of the acceleration in division rate seen in *Paramœcium* and in other cells and tissues.

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